

Review

# Direct and Endophytic Effects of Fungal Entomopathogens for Sustainable Aphid Control: A Review

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**Abstract:** Entomopathogenic fungi (EPF) are cosmopolitan species of great interest in pest management due to their ability to cause epizooty in soil-dwelling and aboveground insects. Besides their direct effect against a wide host range of serious agricultural insect pests, such as aphids, a major emphasis has been placed on investigating the impact of EPF with endophytic traits (EIPF) on aboveground tripartite interactions between host plants, herbivores and beneficial insects. However, despite their valuable role in biocontrol processes, there is still more to explore about their diverse potential as ecofriendly biological control agents. Herein, we provide an overview of the meaningful role and faced challenges following the use of EPF and EIPF to control aphids.

**Keywords:** aphids; hypocrealean fungi; microbial control; multitrophic interactions; volatile organic compounds



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## 1. Introduction

Aphids (Hemiptera: Aphididae) are among the most devastating insect pests in agricultural crops [1]. They are sap-sucking pests, vectors of economically important phytopathogen viruses leading to serious yield losses in various productive cash crops (e.g., sugar beet, sugar cane, potato and wheat) [1,2]. Control of aphids has predominantly relied on the application of synthetic pesticides, including neonicotinoids [3–5]. This practice has generated increasing concern due to the adverse effects on pollinators and other non-target organisms [6–8]; the environment by contaminating all types of soils, including grassland sites [9]; and resistance development among aphid colonies [10]. This series of environmental issues led to the limitation of the use of major commercialized neonicotinoids (clothianidin, imidacloprid and thiamethoxam) by the European Commission in 2018 [11]. Therefore, interest in finding alternative methods to control pests in several crops is of major importance [12,13].

“Microbial control” is a term applied when microorganisms such as viruses, bacteria and fungi are used to control pest insects. Within the aphid management context, due to their characteristic of invading through the cuticle, and thus no need to be ingested, fungi have been found to be important pathogens of aphids [14]. The term “Entomopathogenic fungi” (EPF) is used when the association between fungi and insect leads to a disease in the insect host. Within the fungi kingdom, aphid fungal pathogens are mainly placed in two major clades: Ascomycota (order Hypocreales) and Entomophthoromycota (orders Entomophthorales, Neozygiales and Basidiobolales) [15–17].

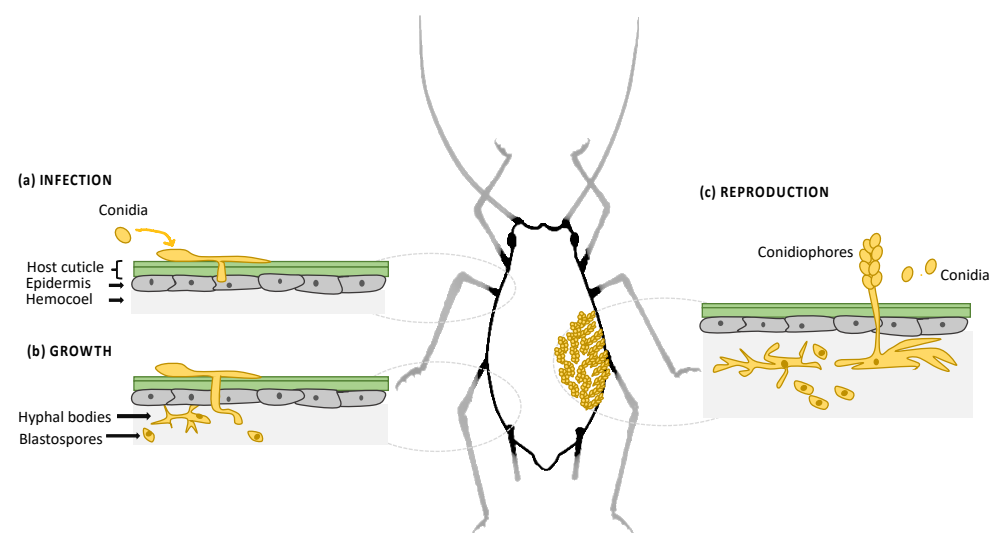
Some of the insect-pathogenic fungi species, mainly within the Hypocreales, are able to live in plant tissues without causing any symptoms to their host, while retaining their killing potential against insects [18]. Indeed, besides their great potential as entomopathogens, the role of endophytic insect-pathogenic fungi (EIPF) in aboveground interactions between plants, herbivores, phytopathogens and auxiliary insects (predators and parasitoids) has been previously reported [18,19]. Their potential as plant disease antagonists, plant growth

promoters and rhizosphere colonizers has been previously highlighted [19]. Thus, it seems conceivable that the direct pathogenic effect against the target insects and indirectly through the plant could occur simultaneously. Such potential would, therefore, have an impact on insect-borne plant pathogens and their vectors. Whereas the importance and role of EPF and EIPF in underlying microbe–plant insect interactions have been recently reported, there is little information about their effects on aphid-borne diseases and also on higher trophic levels, including beneficial insects. In this review, we analyze previous studies based on the direct and indirect effects via endophytism of EIPF against insects, especially aphids. We also discuss the different modes of action that have been suggested and, finally, we highlight some cases of multidimensional interactions reported in the literature.

## 2. Fungal Entomopathogens to Control Aphids

### 2.1. Infection Cycle

The EPF infection path in insect hosts is ensured through a series of subsequent phases [20]: (1) adhesion of a fungal propagule (usually conidia) to the cuticle due to the hydrophobic nature of the fungal feature and specific recognition systems (e.g., lectins); (2) germination of the infectious propagule under suitable environmental factors, including temperature and humidity, with the simultaneous production of extracellular hydrolytic enzymes (proteases and peptidases, chitinases, lipases and lipoxygenases) to exploit host-derived molecules; (3) production of appressoria (differentiation phase) acting as the anchor point on the cuticular surface; (4) penetration of the infective filament by mechanical and enzymatic pressure to the hemocoel; growth, multiplication and formation of hyphal bodies; invasion of tissues; synthesis of toxic metabolites interfering with the host's immune defense mechanisms and death of the infected host (Figure 1). At the end of this process, the fungus emerges from the host cadaver and sporulates. The newly produced asexual conidia are passively dispersed into the environment, causing, at suitable environmental conditions, an epizooty in insect pest populations [21].



**Figure 1.** Illustrative schema of the infection path of hypocrealean pathogenic fungi (case of *Beauveria bassiana*) in an arthropod host (case of aphids). (a) Infection: presented by the conidial attachment, germination, production of appressorial structures and penetration of the host. (b) Growth: described by the fungus' proliferation through hyphal bodies and blastospores, as well as secondary metabolite production in the hemocoel. (c) Reproduction: presented by the fungal outgrowth, where the asexual conidia are passively released and a new cycle of infection is initiated.

### 2.2. Host Immune Response to EPF Infection

There are few studies using sap-sucking hemipteran insects such as aphids to document the key players in the host immune response to fungal infection [22–24]. The genetic

potential of EPF and the resistance levels of their hosts play a key role in defining the infection outcome, represented by fungal growth, host barrier penetration and resistance or not to the enzymatic activity [25]. Studies investigating arthropod defense mechanisms against fungal infection have reported the activation of signaling pathways and the production of antimicrobial peptides (AMP) and lysozymes, leading to the degradation of the EPF [22,26]. Interestingly, aphids have incomplete immune systems, missing essential genes encoding for AMP and other immune-related molecules, and their defense mechanism relies, mostly, on hemocyte recruitment and the activation of phenoloxidase (PO) [22,24,26].

Melanization and encapsulation are two fundamental host mechanisms used through cuticular, cellular and humoral responses to resist fungal infection [25,27,28]. The cuticle is the first barrier that the fungus has to go through, where cuticular phenolic compounds are involved in the formation and deposition of melanin [28,29]. This reaction is elicited either during the penetration process of fungal structures (as hyphae or appressorium) or by the presence of  $\beta$ -1,3-glucans in fungal cell walls [25]. Such a response makes the cuticle more resistant to fungal enzymes. However, most of the entomophthoralean fungal structures are able to either breach the melanin or to penetrate the cuticle before the establishment of the melanization process due to their ability to grow rapidly. The toxic activity of antifungal molecules is another important feature of the humoral response, which may prevent host lethal infection [27,30]. Once the fungus breaks the cuticle, the humoral and cellular responses are activated. The cellular response is initiated by the aggregation of the hemocytes (granulocytes, plasmatocytes) around the fungal bodies, leading either to the phagocytosis process or nodulation and encapsulation, in which the large fungus unit may be lysed [30]. Encapsulation is often accompanied by the activation of cascades of PO, leading to the melanization of the capsule in a melanic sheath [30]. A recent transcriptional regulation study performed on cotton aphid infected with *Beauveria bassiana* showed the significant upregulation of melanization in the hemocoel compared to phagocytosis at the early stage of infection [24]. For the hypervirulent fungal strains, in the case of most obligate entomophthoralean fungi, two situations may occur, where the fungus could either overcome the encapsulation and continue to grow, or the host is not able to form nodules (granuloma). Humoral and cellular defense are likely ineffective against protoplast-growing fungi. For example, fungal species within the genera *Entomophthora*, *Entomophaga*, *Erynia*, *Neozygites* and *Pandora* are able to evade insect hemocytes. In fact, the lack of cell walls and thus of  $\beta$ -1,3-glucans on the surfaces of protoplasts renders the insect host unable to elicit an immune response, since there is no activation either of the PO or the hemocytes [31].

In general, the success of the infection, leading to the host's death, is mainly caused by the presence of fungal toxic metabolites inside the host. For example, upon reaching the hemocoel, *B. bassiana* secrete toxic compounds such as beauvericin, bassianin, bassianolide and oosporein, leading to the death of the host [32]. However, unlike hypocrealean fungi, entomophthoralean fungi exhibit a limited capacity to produce toxins [33]. As an example of an entomophthoralean-produced metabolite, an investigation of pathogenicity-associated genes in *Conidiobolus obscurus* reported the overexpression of a gene encoding for the cytolytic (Cyt)-like endotoxin and serine proteases (subtilisin and trypsin) in infected aphids [23]. Further studies need to be performed on a wide range of model organisms to fill knowledge gaps related to host–pathogen interactions.

### 2.3. Host Range and Potential

Fungal species within the order Entomophthorales, such as *Pandora neoaphidis*, *Entomophthora planchoniana* and *C. obscurus*, and Neozygitales, such as *Neozygites fresenii*, are aphid-host-specific, with high potential to regulate aphid populations in fields and with no side effects on non-target organisms [14,34]. Despite their highly infectious potential, the application of these obligate fungi for aphid control remains difficult in practice because of problems related to the mass production and formulation of the fungal inoculum [35]. In fact, infection cycle complexity from cuticle contact to sporulation, with high depen-

dence upon climatic conditions, but also a lack of efficient mass-scale production allow us to explain their limited use to substitute neurotic insecticides [34]. Recently, an attempt towards biomass production with *Pandora* sp. has been performed by studying biomass production according to different fermentation media [36]. The same study provided a promising result by selecting the media on which high biomass production of *Pandora* sp. was generated [36]. Such a finding is of great interest since it paves the way towards the large-scale production and formulation of this obligate fungal pathogen. However, more studies need to be performed on this aspect, and conservation biological control remains the most adopted strategy when using entomophthoralean fungi in insect pest management [37].

For the hypocrealean fungi, both *Beauveria* and *Metarhizium* genera have been progressively introduced as alternative control agents to control pests. Fungal species such as *M. anisopliae* are generalists with broad insect host ranges, while others, such as *M. acridum*, are often restricted to members of one insect order. Both *Lecanicillium lecanii* and *B. bassiana* (Balsamo) Vuillemin have been tested also to control sap-sucking pests and were proven to negatively affect aphid population growth [38]. Within the aphid management context, the most studied soil-borne fungi are within the genera *Beauveria*, *Metarhizium*, *Verticillium* and *Pacilomyces*. They have been mass-produced, formulated and commercialized against aphids [17]. Species such as *Verticillium lecanii* (Zimmermann), *B. bassiana* and *M. anisopliae* were successfully registered for use in greenhouses against aphids [39]. More research studies have been conducted on these fungal species due to their ability to colonize different types of soil and persist in different environments, and they are considered valuable assets in agriculture [40–42]. Indeed, due to the formulation with synthetic materials, features such as the persistence as well as the infectivity of EPF can be preserved and improved. In addition, the fact that EPF is capable of infecting its host transcutaneously is a major advantage that explains its great interest in agriculture against various host ranges of insect pests [42].

### 3. Fungal Entomopathogens with Endophytic Traits

#### 3.1. State of the Art

EIPF are naturally occurring in several host plants, forming a mutualistic interaction with their host [18]. They live, for at least part of their life, within plant intercellular spaces, tissue cavities and/or vascular bundles, without harming the plant host tissues. Based on six criteria, namely the host range, colonized tissue, in planta colonization, in planta biodiversity, transmission and fitness benefits, endophytic fungi are classified into four types [43]. Multifaceted interactions occur between endophytic fungi and plants and affect plant development and physiological prospects [44].

The role of the fungal endophyte treatments against aphids in limiting their fitness and life cycle has been documented in several studies. Apart from their taxonomy and phylogeny, most of the research studies related to EPF have focused either on their development as biological control agents [45] or their mode of action [46]. Some EPF may colonize plants [46] and the rhizosphere [47,48], extending beyond direct fungi–insect interactions, which requires more investigations to better understand the EPF ecology [48].

As an example, fungi isolated from soybean plants indicated that endophytism was established with the occurrence of *B. bassiana* in all aerial plant parts, while *M. brunneum* was only recovered from stems. Moreover, *Metarhizium* was mainly found to almost exclusively colonize plant roots and was not identified either in the stem or in the leaves of host plants, while *Beauveria* was localized within multiple plant tissues, mainly in the aerial parts [49,50]. The endophytic capacity of these EPF to colonize a diversity of crops, such as banana, maize, cotton, fava bean, poppy, tobacco and wheat, among others, is well documented [51]. Only a few EPF have been studied for their endophytic lifestyle and their plant host ranges, and their potential remains unclear. *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys* and *Isaria* have been isolated from coffee, where they were, most probably,

remaining in the plants as endophytes [52]. Moreover, *Lecanicillium* spp. has been isolated from date palm [53].

Both direct and indirect effects are conditioned through host plants as physical support or chemical machinery. Recently, a meta-analysis study revealed that a reduction in herbivore fitness has often been reported in insects when settled on inoculated plants, regardless of insect taxa, feeding guilds and plant families [54]. Some EPF exhibit multifunctional lifestyles with varying degrees of insect pathogenic and plant endophytic modes [55], but their ecology and mechanisms of interaction until symbioses are still not fully understood. Several EPF have a broader ecology to be associated with potential effects on pest behavior and performance when living within plants. Even if plant colonization was already found to change according to fungal species and strain, environmental conditions and host species [56], further investigations are needed to assess the direct versus indirect effects of endophytic entomopathogens on both plant and insect herbivores in order to more fully explore their potential use in managing insect pests [57].

### 3.2. Pathways of Insect Pathogenic Fungi as Endophytes

Different plant colonization pathways of EPF are described among the investigated diversity of fungi and plant species. Firstly, conidia can initiate colonies in the plant leaves after spraying [52,58]. Foliar spraying showed the highest *B. bassiana* colonization frequency [59], while the leaves of some plants had deficient entry routes for EPF [60]. Moreover, the systemic spread of endophytic fungal colonization varies according to fungal and plant species, leading to a 100% fungal occurrence in treated leaves, compared to 20–40% of the leaf sections in unsprayed leaves from treated plants [61]. Secondly, stem injection is another method for the endophytic colonization of a variety of plants [62]. Thirdly, soil inoculation through roots or seeds was found in several plants, mainly with *Metarhizium* species [50], but also with *L. lecanii*, even if no systemic plant colonization was observed [57]. Seed application of EPF was found to affect insect herbivory above ground. *B. bassiana*, *M. brunneum* and *M. robertsii*, in the seed inoculation of wheat and bean, reduced the population growth of aphids [63]. These fungi were found to confer plant protection [64,65], providing longer-term pest control and establishing intricate interactions in tritrophic contexts. Even if *Beauveria* and *Metarhizium* have been well studied and used as a general model to identify genes involved in insect infection processes, we currently do not know which genes are involved in EPF insect infection in plant colonization. Only a few studies have focused on the production of fungal secondary metabolites in plants as an assumed mechanism, but it has recently been repeatedly suggested that EPF are likely to be responsible for the modulation of the plant's chemical machinery, such as changes in the production of compounds related to plant defenses [54,66]. The effects of EPF inoculations against aphids were hypothesized to be most probably associated with the systemic regulation of the plant's metabolism [63] and should be checked. Further data to support the notion of systemic, rather than local, effects of plant-associated EPF should be generated.

### 4. Mode of Action of EIPF

Besides their direct effects against insect hosts, starting from spore attachment to cuticle, germination, body penetration and colonization by hyphae, leading to death within a few days [67], EIPF exhibit more complex mechanisms in plant pest suppression or tolerance, including the production of fungal metabolites that may act as antibiosis substances, triggering plant-induced systemic resistance (ISR) against pests [19,64,68]. Previous studies on aphid–EIPF interactions have reported an increase in the mortality rate of *Aphis gossypii* feeding on plants tissues colonized by *L. lecanii* or *B. bassiana* [38,57,69]. The same studies suggested that aphid mortality is most likely explained by the direct contact with the spores developed from the extensive EIPF colonization of plant tissue and/or due to the accumulation of fungal metabolites in planta. For example, the ethyl



acetate fractions extracted from the culture filtrate of *L. lecanii* caused significant aphid mortality [38].

The mechanisms by which aphids can be negatively affected by EIPF have also been studied in a few different grass species and effects were mediated by the constitutive production and/or induction of secondary compounds produced by the plant or secondary metabolites produced by the endophytes themselves [70]. Specifically, the release in plants of several enzymes associated with insect body degradation was thought to be one part of the EIPF ability to control the pest population by developing these toxic enzymes [67]. Several non-mutually exclusive mechanisms have been suggested to explain these effects [64]. Other studies have attributed the negative effects on aphid fitness to systemic chemical changes induced by the endophyte's presence, without clear evidence of specific mechanisms in endophytically colonized plants [67,70]. Some EIPF were found to alter the phytosterol profiles of plants and compete with insects for these compounds, which are essential for their development [71]. Other compounds, namely alkaloids, indole-diterpenes, sesquiterpenes and diacetamides, were already examined for their negative effects on pests and corresponded to extractions from fungal endophytes or endophyte-infected plants [72,73]. Moreover, benzoxazinoids and flavonoids were identified in host plants in response to EIPF inoculation and associated with a reduction in aphid infestation, helping to elucidate the role of specific plant secondary metabolites in plant–fungus–herbivore interactions [63]. Finally, the accumulation of mycotoxins in plant tissues colonized by EIPF, such as *L. lecanii*, is another example of a reduction in aphid survival rates [74]. Antibiosis due to the production of fungal secondary metabolites within plants has been previously suggested to be the main mechanism of action of endophytic entomopathogenic fungi in several studies [64]. The activation and transport of defensive compounds was identified, including in the phloem, to cope with aphids [75].

A second mechanism is related to antixenosis induced by EIPF plant colonization and, more particularly, in the changes in volatile organic compounds (VOCs) and further effects to disturb pest behavior and reduce plant damages. Epichloe–grass symbiosis was described as an EIPF-mediated VOC response that occurred through plants, with negative effects on aphids [76]. Several behavioral responses in insects (foraging, finding mates and oviposition sites and avoiding natural enemies) are mediated by olfactory chemical cues emitted by plants. The endophytic colonization of plants has been reported to correspond to EPF's effect of modifying the volatile emissions and the fact that microbial volatiles may modify plant–insect interactions [77,78]. Endophytic EPF was determined to deter aphid consumption [79]. A few studies dealing with the impact of EIPF on host plant selection behavior by pests highlighted that qualitative and quantitative differences in the blend of volatiles released by endophytically colonized leaves were associated with changes in insect behavior [61].

## 5. Illustration of the Potential of EPF and EIPF on Aphid Fitness and Performance

Around 170 mycoinsecticides developed from at least 12 species of EPF, including species within the genus *Beauveria* [19]. They are able to suppress pest populations and persist in the environment. Most of them are applied in agriculture by inundative methods to control insects, mites and ticks [19]. Within an integrated pest management (IPM) context, the combination of different control agents often is found to increase the efficacy and longevity of the individual technologies, e.g., the chemical compound azadirachtin with *B. bassiana* decreased the populations of both the rice root aphid *Rhopalosiphum rufiabdomianale* (Sasaki) and the honeysuckle aphid *Hyadaphis foeniculi* (Passerini), in celery [80]. Such promising findings pave the way for the implementation of innovative and sustainable eco-compatible approaches in IPM by combining different effective control agents, such as plant-growth promoting rhizobacteria [81], semiochemicals [82] and EPF.

Herein, the potential of different fungal species, following direct and indirect treatments, on a wide range of aphid species have been summarized (Table 1). In most of the studies dealing with the entomopathogenic potential (direct effect) of fungi against aphids, the main focus has been allocated mainly to studying the mortality, and a few reports on the aphids’ fitness and behavior. This is mainly due to the fact that when aphids are treated with EPF, the infection process leads to the early mortality of individuals, making it challenging to monitor other parameters that require more time, notably reproduction. This was reported previously when treating the target *Acyrtosiphon pisum* with a sub-lethal dose of *P. neoaphidis* and *B. bassiana* and assessing the impact of such treatment on aphid nymph production [83]. However, for the assessment of the endophytic potential of fungi (indirect effect), it is feasible to consider several parameters. The level of impact varies very little for parameters such as mortality and reproduction. Most of the studies have reported that, regardless of the mode of application, EPF has a very high impact on mortality and reproduction. The variation in the level of impact for the population growth parameter is probably related to the fact that there have been very few studies that have tested this parameter [74,84].

**Table 1.** Synthetic list of studies on entomopathogenic fungi and target aphid species. Level of impact on the studied parameters (mortality, reproduction, population growth and behavior) is represented by different color codes: red for high; orange for moderate; yellow for low; white for not tested.

Fungal Species	Aphid Species	Mortality	Reproduction	Population Growth	Behavior	References
<b>Direct</b>						
<i>Beauveria bassiana</i>	<i>Aphis gossypii</i>	High	High	Not tested	High	[38,85]
	<i>Acyrtosiphon pisum</i>	High	High	Not tested	Low	[83]
	<i>Brevicoryne brassicae</i>	High	High	Not tested	Not tested	[86]
	<i>Lipaphis erysimi</i>	High	High	Not tested	Not tested	[86]
	<i>Megoura japonica</i>	High	High	Not tested	Not tested	[67]
	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85,87]
	<i>Rhopalosiphum padi</i>	High	High	Not tested	Not tested	[86]
	<i>Schizaphis graminum</i>	High	High	Not tested	Not tested	[86]
<i>Cordyceps scarabaeicola</i>	<i>Aphis gossypii</i>	High	High	Not tested	Not tested	[85]
	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85]
<i>Cordyceps</i> sp.	<i>Myzus persicae</i>	Low	Low	Not tested	Not tested	[87]
<i>Isaria</i> spp.	<i>Myzus persicae</i>	Low	Low	Not tested	Not tested	[87]
<i>Lecanicillium lecanii</i>	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85]
	<i>Aphis gossypii</i>	High	High	High	High	[38,85]
<i>Lecanicillium</i> spp.	<i>Myzus persicae</i>	Low	Low	Not tested	Not tested	[87]
<i>Metarhizium anisopliae</i>	<i>Aphis gossypii</i>	High	High	Not tested	Not tested	[85]
	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85]
<i>Metarhizium brunneum</i>	<i>Myzus persicae</i>	High	High	High	Not tested	[88]
<i>Metarhizium majus</i>	<i>Myzus persicae</i>	Low	Low	Not tested	Not tested	[88]
<i>Metarhizium pinghaense</i>	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[88]
<i>Metarhizium robertsii</i>	<i>Myzus persicae</i>	Low	Low	Not tested	Not tested	[88]
<i>Nomuraea rileyi</i>	<i>Aphis gossypii</i>	High	High	Not tested	Not tested	[85]
	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85]
<i>Paecilomyces farinosus</i>	<i>Aphis gossypii</i>	High	High	Not tested	Not tested	[85]
	<i>Myzus persicae</i>	High	High	Not tested	Not tested	[85]
<i>Pandora neoaphidis</i>	<i>Acyrtosiphon pisum</i>	High	High	Not tested	Low	[83]

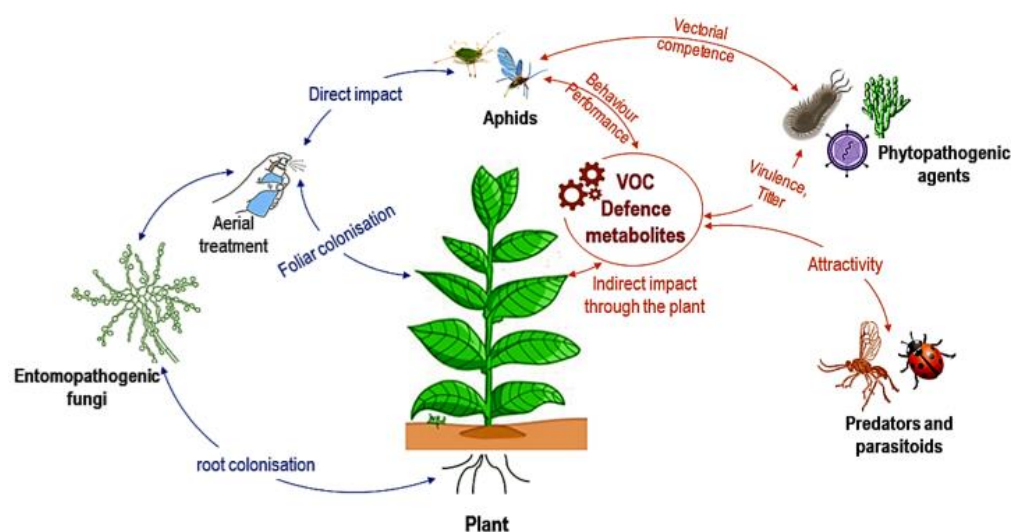
Table 1. Cont.

Fungal Species	Aphid Species	Mortality	Reproduction	Population Growth	Behavior	References
<i>Verticillium lecanii</i>	<i>Acyrtosiphon pisum</i>	■				[89]
	<i>Aphis gossypii</i>					[89]
	<i>Megoura japonica</i>					[67]
	<i>Myzus persicae</i>					[89]
<b>In planta (endophyte)</b>						
<i>Beauveria bassiana</i>	<i>Acyrtosiphon pisum</i>		■	■		[84]
	<i>Aphis fabae</i>		■			[84]
	<i>Aphis gossypii</i>	■	■	■	■	[57,61,70]
	<i>Myzus persicae</i>		■	■	■	[52,74,90]
	<i>Sitobion avenae</i>	■	■			[91]
<i>Epichloe festucae</i>	<i>Aploneura lentisci</i>	■	■			[92]
	<i>Diuraphis noxia</i>	■	■			[92]
	<i>Rhopalosiphum padi</i>	■				[93]
	<i>Schizaphis graminum</i>	■				[93]
<i>Fusarium oxysporum</i>	<i>Acyrtosiphon pisum</i>			■		[84]
	<i>Aphis gossypii</i>			■	■	[94]
<i>Gibberella moniliformis</i>	<i>Acyrtosiphon pisum</i>		■	■		[84]
	<i>Aphis fabae</i>		■			[84]
<i>Hypocrea lixi</i>	<i>Acyrtosiphon pisum</i>			■		[84]
<i>Isaria fumosorosea</i>	<i>Myzus persicae</i>	■		■		[52]
<i>Lecanicillium lecanii</i>	<i>Aphis gossypii</i>		■			[57]
<i>Metarhizium anisoplaie</i>	<i>Acyrtosiphon pisum</i>			■		[84]
	<i>Myzus persicae</i>	■		■		[52]
<i>Metarhizium brunneum</i>	<i>Aphis gossypii</i>	■	■	■	■	[61]
	<i>Myzus persicae</i>		■	■		[74]
<i>Neotyphodium lolii</i>	<i>Metopolophium dirhodum</i>		■	■		[95]
	<i>Rhopalosiphum padi</i>		■	■		[95]
<i>Not identified</i>	<i>Diuraphis noxia</i>				■	[96]
<i>Purpureocillium lilacinum</i>	<i>Aphis gossypii</i>	■	■			[70]
<i>Trichoderma asperellum</i>	<i>Acyrtosiphon pisum</i>		■	■		[84]
	<i>Aphis fabae</i>		■	■		[84]

## 6. Multitrophic Effects of EIPF

Plants, insects and microbes are part of the terrestrial agroecosystem, where underground and aboveground trophic interactions play a key role in shaping the relationships between these players. Since most of the economically important insect pests, such as aphids, are vectors of plant pathogens, treatment with EIPF could have consequences on the spread of these phytopathogens within either insect vectors or plants. Furthermore, predators and parasitoids could also be impacted by the presence of EIPF by modulating their attractiveness towards both plants and their prey (Figure 2).





**Figure 2.** Direct and indirect effects of entomopathogenic fungi treatments on aphids, plants, plant pathogens and insect beneficials in a multidimensional interaction.

However, there is little information describing the impact of EIPF on higher trophic levels, such as parasitoids and secondary parasitoids (i.e., parasitoids of the primary parasitoids; hereafter “hyperparasitoids”). Thus far, the few studies conducted on this topic provide various results, ranging from no effect to a negative effect on parasitoid life history traits [76]. Moreover, little is known about endophytic EPF’s effects on the VOC emissions of host plants and the cascading effects on the behavior of insects at different trophic levels. *Epichloë* endophytes were found to modify both the constitutive and aphid-induced VOC emissions of host grasses. The two studied fescues differed substantially in VOC emission patterns in response to aphid infestation and endophyte infection [76]. Moreover, changes in plant odor led to the increased abundance of natural enemies on endophyte-colonized plants, thereby indirectly affecting plant defense. There is also some evidence that EIPF affects the performance and host selection behavior of parasitoids. For example, parasitized aphid hosts reared on an endophyte-infected model grass (*Lolium perenne*) reduced the lifespan of hyperparasitoids that were able to perceive the disadvantage for their developing offspring in parasitoids from the endophyte environment and could learn to discriminate against them.

As EIPF was found to affect the probing and feeding behavior of aphids, virus transmission was also modified as well. An electropenetrography (EPG) study indicated that *B. bassiana* plant colonization modified the virus acquisition process by aphids and conferred protection against mechanical inoculation viruses [97]. Gonzalez-Mas and colleagues [98] suggested that the mechanism by which *B. bassiana* reduces the virus transmission rate is by inducing systemic resistance in the host plant to viruses.

Even if aphid vector feeding, reproduction and abundance decrease in EPF treated plants, no impact on viral titer has been observed in plants. Endophyte infection alleviated the negative effect of virus infection on the proportion of total plant biomass allocated to roots [99]. Moreover, *T. harzianum*- and *M. anisopliae*-inoculated plants showed reduced severity and titer levels for one virus. Antagonistic effects of EPF endophytes against viruses have also been reported on some plant–virus models: Barley yellow dwarf virus (BYDV) in endophytically infected meadow rye grass and zucchini yellow mosaic virus (ZYMV) in squash [100].

## 7. Molecular Approaches to Enhance EPF Performance

The development of EPF-based insecticides is an effective and sustainable management tactic against agricultural insect pests. Besides their direct effects as entomopathogens, the endophytic traits of most commercialized fungal strains, including plant growth promotion

and induced ISR, add to the benefits in a field setting. However, the efficacy of insect pathogenic fungi still depends on a multitude of environmental factors. Indeed, abiotic factors such as ultraviolet radiation, humidity and high temperatures [101], and biotic ones such as the insect growth stage, occurrence of other microbes and plant host compounds, may impact the efficacy of EPF [102]. Due to the low efficacy of EPF compared to chemical insecticides [101], devoting efforts to performing genomic and transcriptomic studies on EPF will help to select the most virulent fungal strains, leading to the cost-effective application of novel mycoinsecticides. To improve the virulence of EPF, genetic engineering of these microbial actors will offer an opportunity for a deeper understanding of the molecular machinery and the secondary metabolites regulating host–fungal pathogen interactions, therefore improving their tolerance to environmental stress. For example, the upregulation of genes encoding for the endogenous cuticle-degrading protease Pr1 in *Metarhizium* and the CHIT1 gene that encodes chitinase in *B. bassiana* improved the virulence of both fungi [48,103]. Furthermore, transcriptomic studies on insects infected with *B. bassiana* and *Metharizium* spp. revealed the presence of fungal toxic compounds and enzymes such as chitinase, sterol carriers and benzoquinone oxidoreductase, related to the fungal pathogen's genes Bbchit1, Mr-Npc2a and BbBqrA, respectively [102].

## 8. Conclusions

Plant–aphid interactions are complex and should be investigated through a broadening approach, including more systematically associated microorganisms. While EPF were first selected for their direct toxic effects on aphids, recent work on the endophytic behavior of some of these fungi indicates that they can also indirectly control aphids and associated viruses through priming for enhanced plant ISR. Furthermore, this would decrease the need for pesticide use, thus reducing off-target effects on beneficial insects in the environment. Hence, further investigations are needed to understand the EPF/EIPF–plant interactions and the active mechanisms in terms of the biology and behavior of aphid pests and non-target organisms, including predators and parasites, to ensure efficient biological control measures. Including microbials such as EPF/EIPF in multitrophic interactions will allow us to reduce conventional pesticide applications and promote integrated pest management with an efficient and sustainable approach.

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